REMARKS

Claims 18-34 are in this application. Claims 18-34 are new. Claims 1-17 are cancelled. Claims 18-34 correspond substantially to the previous version of claims 1-17 and are supported by those claims.

In view of new claims 31 and 32 that define the kit as a kit for performing the method of claim 18, it is respectfully requested that these claims be examined in this application.

According to the action, claims 1-13 and 16-17 are rejected under 35 USC 112, second paragraph. This is respectfully traversed.

In view of new claims 18 and 24, this rejection is moot and it is respectfully requested that the rejection be withdrawn.

According to page 6 of the action, claims 1, 3-5, 7, 10, 12-13 and 16-17 are rejected under 35 USC 102(b) as being anticipated by Donnelly et al. Claim 2 is also rejected as being obvious in view of this reference in combination with Fernandez et al., 2000. Claim 6 is rejected as being obvious over Donnelly et al. Claims 8-9 are rejected as being obvious over the combination of Donnelly et al., in view of Revel et al. or Fernandez et al., 2003. Claim 11 is rejected as being obvious over the combination of Donnelly et al. in view of Kruger et al. These are respectfully traversed.

According to the abstract of Donnelly et al. "The aim of this study was to determine sperm nuclear integrity and mitochondrial function, to quantify possible apoptosis and to investigate any relationship between these parameters....DNA integrity was determined using a modified alkaline single cell gel electrophoresis (Comet) assay. DNA fragmentation, possibly indicative of apoptosis, was detected by terminal deoxynucleotidyl transferase mediated dUTP nick end labelling (TUNEL)."

In Donnelly, the cells were lysed with a cold lysis solution (NaCl, Na_2EDTA, Tris and Triton X-100) for 1 hour at 4^0C. Later cells, were incubated for 30 minutes at 4^0C in DTT, followed by incubation for 90 minutes at 20^0C with lithium diiodosalicyclate (LIS) in order to decondense the DNA (see third paragraph in the left hand column on page 1554 of Donnelly.)

Anticipation requires that each and every element of the claimed invention be disclosed in a single prior art reference. *In re Paulsen*, 30 F.3d 1475, 31 USPQ 1671 (Fed. Cir. 1994). For anticipation, there must be no difference between the claimed invention and the reference disclosure, as viewed by a person of ordinary skill in the field of the invention. *Scripps Clinic & Res. Found. v. Genentech, Inc.*, 927 F.2d 1565, 18 USPQ2d 1001 (Fed. Cir. 1991).

Clearly, given the differences between Donnelly et al. and claim 1, Donnelly does not anticipate claim 1, now claim 18.

Claim 18 requires a single lysis step (step b) as compared to the two sequential steps of Donnelly et al. As disclosed in the first column on page 1554 of Donnelly et al, two lysis steps are carried out to evaluate the DNA integrity: EDTA, Tris and Triton are used as lysis agents for the first lysis step and DTT is used for the second lysis step.

Claim 18 defines that the evaluation stage of the integrity of the chromatin/DNA of the sperm is based on the measurement of the halo size of the sperm cells (step c).

Although not required by claim 18, the sample, after treatment steps a) and b), can be stained and the halo size can be measured using conventional microscopy. In contrast, Donnelly et al., discloses that DNA fragments are separated by electrophoresis before being stained with fluorochromes to develop the Comet assay and subsequently the captured images are analyzed using fluorescence microscopy and an image analyses system software (page 1554). Donnelly et al. does not suggest nor disclose that after the Comet assay, measurement of halo size. In a Comet assay, the fragmented spermatozoids are visualized as a tail of fragments (comet tail) migrating in the electrophoresis direction. There is no halo to be evaluated.

Therefore, since there are features of claim 1 that are not disclosed in Donnelly et al., it is respectfully requested that the rejection of claim 1 and the claims that depend thereon, namely claims 3-5, 7, 10, 12-13 and 16-17 as being anticipated by Donnelly et al. be withdrawn.

The obviousness rejections based on Donnelly et al., should also be withdrawn.

As explained above, different methods for evaluating the integrity of chromatin/DNA of human sperm are known. Two of these methods, TUNEL and COMET are disclosed in Donnelly et al. Features and disadvantages of these methods are disclosed on page 2, lines 21-35 and page 3, lines 1-10 of the application. In the method of this invention, these disadvantages are overcome.

As described above, the method of claim 1 differs from Donnelly et al. in that it simplifies the lysis step to just one single step and in the evaluation stage the integrity of the chromatin/DNA of the sperm is based on the measurement of the halo size of the sperm cells which can be done using a conventional microscope, and without an electrophoresis step. The claimed invention results in a process that is more reliable than the method of Donnelly et al. This is due to the use of a single lysis step and evaluation of the integrity of the chromatin/DNA of the sperm based on the measurement of the halo size of the sperm cells.

The use of a single lysis step has the effect of not affecting negatively the structure of the spermatozoids. This permits identification of the sperm cells with respect to other kind of cells that may be present in the sample. The use of a single lysis step also has the advantage that quality and contrast of the images obtained is very much improved. Therefore, more reliable results can be obtained with improved reproducibility.

Due to the use of a single lysis step, the fact that electrophoresis is not required and halo visualization is used results in a technique that is simpler, faster and less expensive.

As described in the subject application and as can be seen in figures 1 and 2 attached herewith, the features discussed above such as the use of a single lysis solution, result in a less aggressive treatment which typically results in:

1) keeping the tails of the sperm cells. Keeping the tails of the sperm cells is a very important improvement over the known processes. Their identification is morphological data to distinguish if the images of nucleoids really correspond to sperm cells or to other types of cells that could be present, for example, desquamated cells of the genitourinary tract, inflammatory cells, blood cells etc.

2) the improvement in the quality of images of nucleoids, that can be viewed with the necessary precision with, for example, a conventional bright field microscope. In Donnelly et al. the sperm cells are stained with fluorochromes, and a fluorescence microscope is needed to view them. The single lysis step of the claimed invention achieves unfolding of the chromatin loops resulting in better maintenance of the morphology of the previous head or core and obtaining dispersion halos with a greater density of chromatin material. This enables that the halos with, for example, the Wright or Diff-Quik dye, can be viewed under a conventional bright field microscope. Consequently, the contrast and visual discrimination of the different sizes of halos is possible.

The staining techniquies using Wright dye or Diff-Quik are among the simplest, cheapest and most routine ones in any laboratory. These stainings can be easily manipulated for obtaining the intended staining level.

Finally, the single lysis step of the invention permits the use of smaller quantities of reagents. For example. DTT is costly and the reduction to the concentration described (a fourth of that described in Donnelly et al. (see claims 22 and 32) is enough.

Use of the method of this invention, permits one to determine if the nucleoids come from mature sperm cells or other cell types.

Fernandez 2000 describes two alternatives for in situ DNA breakage detection. In the first, cells were initially incubated in the alkaline unwinding solution for transformation of DNA breaks into single-stranded DNA (ssDNA) to be hybridized, followed by lysing solutions for protein removal. In the second, incubation in the lysing solutions was carried out before the denaturation step.

Revel describes the use of Resveratrol, a natural aryl hydrocarbon receptor antagonist, as a lung protector from DNA damage and apoptosis caused by benzo[a]pyrene.

Fernandez et al. 2003 cited in the present application, described a technique which enabled the chromatin of human sperm to be dispersed in situ, demonstrating that those sperm incapable of dispersing the chromatin contained fragmented DNA. Samples of semen are treated sequentially in agarose microgel with an acid denaturing solution, with two lysis solutions and with a wash.

None of these references alone or in combination disclose a method to evaluate the integrity of DNA using a single lysis step and measurement of integrity of the chromatin/DNA of the sperm based on the measurement of the halo size of the sperm cells. In light of the content of these documents, one skilled in the art would not have combined these with Donnelly et al. and would not have a reasonable expectation of success.

Therefore, it is respectfully requested that these obviousness rejections be withdrawn.

It is submitted that the application is in condition for allowance and favorable consideration is respectfully requested.

Respectfully submitted,

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